the EBV+ tumor were selected. A total of 25 lines were infused. We evaluated HLA restriction in 20 of these lines and inferred it in five. CTLs were restricted by a single HLA allele (n=12), by two alleles (n=6) and less frequently by > than two alleles (n=2).
Patients received a median of 6 infusions most at $1x10^6$ EBV-CTL/kg. Five patients received EBV-CTLs from >1 3rd party donor. Infusions were associated with minimal toxicity. Sixteen patients achieved CR, two patients PR and two SD. Eight patients died of progressive disease, five shortly after the first infusion (17- 29 days). Response to EBV CTL therapy correlated with HLA restriction but not the degree of HLA matching. Radiographic and clinical responses correlated with detectable increases in the frequency of CTL precursors in the blood. However durable EBV CTL engraftment was not seen.
This study demonstrates a high response rate among patients with otherwise refractory EBV malignancy treated with EBV specific 3rd party CTLs. EBV CTLs can be effective when selected based on restriction to shared alleles despite significant HLA disparity. The bank of EBV specific T cells can provide an immediate source of HLA partially matched appropriately restricted T cells for adoptive immunotherapy to treat EBV associated malignancy. This enables treatment early in the course of disease and is anticipated to maximize the response rate.

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Clinical Trial Evaluating DC/AML Fusion Cell Vaccination in AML Patients Who Achieve a Chemotherapy-Induced Remission

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We have developed a promising leukemia vaccine in which patient derived AML cells are fused with autologous dendritic cells (DCs), presenting a broad array of antigens. We are conducting a clinical trial in which AML patients undergo vaccination with DC/AML fusion cells following chemotherapy induced remission. Twenty-six patients underwent collection of AML cells at disease presentation for vaccine generation and immune monitoring studies. Median age of the patients is 66 years. Tumor was collected from either a bone marrow aspirate (N=16), 20 cc of peripheral blood (N=7), or leukapheresis product (N=3) at the time of presentation with newly diagnosed AML (N=25) or first relapsed AML (N=1). The mean yield of AML cells was 109 x 10^6 cells with a mean viability of 91%. Eligible patients achieving CR (N=16) underwent leukapheresis for DC generation. Adherent peripheral blood mononuclear cells were cultured in the presence of GM-CSF and IL-4 for 5-7 days, and exposed to TNFα for 48-72 hours to generate mature DCs. Fusion cells were generated by co-culture of DCs with AML cells in the presence of 50% polyethylene glycol and identified as cells co-expressing antigens that were unique to the DC and tumor cells. Mean fusion efficiency and viability was 38% and 85%, respectively. Vaccination with DC/leukemia fusion cells was initiated within 12 weeks from count recovery following the final cycle of chemotherapy. 13 patients received at least two monthly vaccinations at a dose of $5x10^6$ fusion cells. 8 patients had intermediate risk cytogenetics, 3 patients had good risk cytogenetics, and 2 patients had a complex karyotype. Vaccination was well tolerated, and importantly, was not associated with clinically significant auto-immunity. Biopsy of vaccine site reactions demonstrated a dense infiltrate of CD4 and CD8 T cells consistent with recruitment of reactive T cell populations to the vaccine bed. To date, 9 patients remain in remission (69%), with a mean follow up of 23 months. Peripheral blood samples were collected prior to each vaccination and at 1, 3, and 6 months following completion of vaccination. Vaccination resulted in the potent induction of leukemia specific immunity as measured by an 8 fold increase (mean, n=6) in CD8 T cells expressing IFNγ in response to ex vivo exposure to autologous leukemia cell lysates. Bone marrow derived T cells were isolated prior to and following vaccination in patients who are HLA2.1+. Vaccination resulted in the expansion of bone marrow infiltrating T cells recognizing MUC1 (9 fold increase), WT1 (5 fold increase), PRAME (12 fold increase) tumor antigens by tetramer analysis (n=2). In conclusion, DC/AML fusion cell vaccination results in the potent expansion of leukemia reactive T cells and durable remissions following chemotherapy. Enrollment to a second cohort in which patients receive DC/AML fusion cell vaccination in combination with antI-PD1 antibody is planned.

GVH/GVL

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In Vivo T Regulatory Cell Kinetics Are Altered in a Pre-Clinical Model of Chronic Graft-Versus-Host Disease

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Chronic graft-versus-host disease (cGVHD) is the main cause of late morbidity and non-relapse mortality after allogeneic hematopoietic stem cell transplantation (AHSCT). T cells are known modulators of cGVHD, while their *in vivo* kinetics, as defined by cell division, cell death and trafficking, remain largely uncharacterized, and current therapies for cGVHD target T cell proliferation. T regulatory cells (T regs) are of particular interest in cGVHD as adoptive transfer or *in vivo* expansion of this cell type has resulted in amelioration of cGVHD severity in pre-clinical models and in patients with cGVHD. We have developed a method to measure T reg and non-T reg cell kinetics using deuterated water labeling/de-labeling combined with triple quadrupole GC/MS detection of deuteurium enrichment in DNA of dividing/dying cells. Using a minor antigen mismatch murine model of cGVHD we show that in AHSCT recipients, donor CD4+ non-T regs in the spleen have a proliferative advantage over T regs, while in the syngeneic (genetically matched donor-recipient pairs) control animals cell gain rates for these populations are similar. The net result of the differential cell kinetics in the cohort with cGVHD was fewer T regs in the spleens of allogeneic versus syngeneic marrow recipients, with a suggestion for decreased trafficking out of the spleen. Evaluation of T reg and non-T reg CD4+ T cells within lymphoid compartments (lymph nodes and peripheral blood) and target organs (liver, intestines and skin) allowed assessment